Pending Claims

Prior to the entry of the above amendments, Claims 2-22 are pending. Claims 22 and 2-11 are directed to methods for production of a mutant high alkaline protease; Claims 12-13 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; Claims 14-16 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline protease; Claims 17-18 are directed to a high alkaline protease; Claim 19 is directed to a detergent composition comprising as an active ingredient a alkaline protease; Claim 20 is directed to use of a high alkaline protease in a detergent composition; and Claim 21 is directed to use of a high alkaline protease in a laundry process.

The Office Action

Claims 2-11 and 22 were rejected under 35 USC 112, first paragraph, on the basis that they are not limited to methods of producing a *Bacillus* novo species PB92 of reduced indigenous extracellular protease level, transformed with a mutated *B.* novo PB92 alkaline protease.

Claims 2, 9, 12, 14 and 17-21 were rejected on the basis that they are vague and indefinite.

Claims 22, 3, 6-7 and 10-11 were rejected under 35 USC 102(b) on the basis that they are clearly anticipated by Bott *et al.*

Claims 2, 4, 5, 8-9, and 14-21 were rejected under 35 USC 103 as being unpatentable over Bott *et al.*, in view of Van Eekelen *et al.*

Claims 12 and 14 were rejected under 35 USC 102(b) as anticipated by, or in the alternative under 35 USC 103, as obvious over Fahnestock *et al.*

Claims 13 and 15-16 were rejected under 35 USC 103 as being unpatentable over Fahnestock *et al.* in view of Van Eekelen *et al.*

These rejections are addressed individually below.

Amendments

Claims 4, 9, 10, 12, 14, 15, 17 and 19 have been amended. Claims 2-3, 8, and 18 have been cancelled. Claims 20-22 have been cancelled and rewritten as new Claims 23-25. New Claim 26 has been added. For the Examiner's convenience, the text of pending claims is attached as Appendix I.

Claims 4-7 and 9-10 have been amended to change the dependency. Claim 4 has additionally been amended to recite that the *Bacillus* strain is a *Bacillus* novo species PB92 or a derivative thereof. The amendment to include *Bacillus* novo species PB92 may be found, for example, in Claim 13 as filed.

Claim 9 has been amended to recite that the high alkaline protease is a mutant and is obtained from *Bacillus* novo species PB92. The claim finds support in Claim 9 as filed and throughout the specification.

Claim 12 has been amended to recite that the vector comprises flanking regions of a gene coding for a high alkaline protease wherein the flanking regions provide for homologous recombination. Support for this language may be found, for example, on page 8, lines 14-19.

Claim 14 has been amended to delete "capable of" as requested by the Examiner. Additionally, the claim has been amended to provide that the protease gene is a high alkaline protease gene and that the strain has been obtained by transforming a mutant alkalophilic *Bacillus* strain according to the method of Claim 12 or 13. Claim 14 has also been amended to recite that the indigenous protease gene is an extracellular alkaline protease gene and that the mutant strain has a reduced indigenous extracellular protease level. This language find support in Claim 12 as filed (see the preamble).

Claim 15 has been amended to change "mutation" to "mutant."

Claim 17 has been amended to recite that the high alkaline protease is produced according to the method of Claim 23 and that it is substantially free from contamination with an

indigenous extracellular alkaline protease. Support for this language may be found, for example, in Claim 12 as filed.

Claim 19 has been amended to change the dependency and to make the claim more readable.

Claims 20-22 have been rewritten as new Claims 23-25 and find support in original Claims 1 and 20-21. New Claim 26 finds support on for example page 12, lines 29-31 and in Claim 1 as filed.

No new matter is introduced by the above amendments, and the Examiner is respectfully requested to enter them.

Ownership

Applicants state for the record that the subject matter of the various claims was commonly owned at the time any invention covered therein was made.

Miscellaneous

The Examiner noted that the dependency in Claim 2 was incorrect. This issue is now moot; Claim 2 has been cancelled.

Section 112, first paragraph

Claims 2-11 and 22 stand rejected on the basis that they are not limited to methods of producing a *Bacillus* novo species PB92 of reduced indigenous extracellular protease level, transformed with a mutated *Bacillus* novo PB92 alkaline protease. This rejection is respectfully traversed because the claims are not directed to methods of producing a strain of *Bacilli*, but, as amended, are directed to a method for production of a mutated high alkaline protease substantially free of indigenous extracellular protease (as amended). Sufficient detail is provided in the specification to enable one skilled in the art to make and use the claimed invention. For example, on page 12 beginning at line 22, is described

methods for producing a mutated high alkaline protease. The method is exemplified in Example 8 beginning on page 27.

In regard to specific claims, Claim 22 has been rewritten as new Claim 23 and uses language suggested by the Examiner to describe the host cell, namely that it has a reduced indigenous extracellular protease level. In regard to Claim 14, "capable of" has been deleted.

In regard to Claim 9, the claim has been amended to delete the language objected to by the Examiner.

In view of the above remarks and the amendments to the claims, the claims are now clearly enabled by the specification and the Examiner is respectfully requested to withdraw this rejection.

Section 112, second paragraph

Claims 2, 9, 12, 14 and 17-21 stand rejected on the basis that they are indefinite. The individual objections are addressed below.

Claim 2 has been cancelled. Claim 9 has been amended to delete the language objected to by the Examiner. Claim 12 has been amended to delete the language objected to by the Examiner. Claim 14 has been amended to delete the language objected to by the Examiner. Claim 17 has been amended to recite that the claimed enzyme is a mutant high alkaline protease which differs in at least one amino acid from a wild-type high alkaline protease and to more clearly differentiate between the claimed enzyme and the one of which it is a mutant.

Claim 18 has been cancelled. In regard to Claims 19-21, Claim 19 has been amended to replaced "proteases" with --protease--. Claims 20-21 have been rewritten as new Claims 24-25 in correct U.S. format.

In view of the above remarks, it is respectfully submitted that the claims as written are definite. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Section 102(b)

Claims 22, 3, 6-7 and 10-11 stand rejected as being clearly anticipated by Bott *et al*. This rejection is respectfully traversed because Applicants have amended the claims to read only on high alkaline proteases and the Bott *et al*. application refers only to serine protease and neutral protease.

The high alkaline proteases of the claimed invention are obtainable from alkalophilic *Bacilli*, defined as those that grow under alkaline conditions, generally pH 9-11. (See page 10, lines 21-29.) Alkalophilic *Bacilli* and high alkaline proteases are not disclosed by the Bott *et al*. reference; accordingly, a method for obtaining high alkaline proteases is not disclosed either.

In regard to the paragraphs quoted by the Examiner, these quotes were taken out of context and used to form mosaic which seems to give the impression that the present invention was already completely disclosed by Bott *et al*. When read in context, however, it is clear that Bott *et al*. does not teach the claimed invention. For example, the first paragraph quoted by the Examiner is "this is facilitated by bacterial strains which are particularly susceptible to homologous recombination. [page 13]." See bottom of page 5, top of page 6, of Paper 6. However, when read in context, this paragraph says only that there exist some bacteria which are particularly prone to homologous recombination. There is nothing new in this remark and it certainly does not render the claimed invention anticipated.

New Claim is not anticipated by Bott *et al*. Bott *et al*. in fact teach away from the claimed invention. For example, on page 2, lines 7-14, it is mentioned that expression of protease genes is "coordinately regulated in concert with sporulation". On page 3, lines 26-30, it is emphasized that asporogenous mutants are "unsatisfactory for the recombinant production of heterologous proteins". The alkalophilic strains of the present invention are asporogenous.

In view of the above remarks and the amendments to the claims, the Examiner is respectfully requested to withdraw this rejection.

Section 103

Claims 2, 4, 5, 8-9 and 14-21 stand rejected on the basis that they are unpatentable over Bott *et al.* in view of Van Eekelen *et al.* As discussed above, there is no teaching in Bott *et al.* regarding methods for making a mutant high alkaline protease, nor is there any disclosure of high alkaline protease obtained according to the claimed method. In regard to the Van Eekelen *et al.* reference, it is a PCT application which claims priority to European Application 87200356.1 filed February 27, 1987. Due to an oversight, Applicants had failed to claim priority to this application and accordingly have attached hereto a Supplemental Declaration (unexecuted) which sets forth a claim under Section 120 to U.S. Application 07/162,105 filed February 28, 1988 and under Section 119 to European Application 87200356.1 filed February 27, 1987. The latter is the same priority document as that recited in PCT/NL88/0006 (Van Eekelen *et al.*). Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Claims 13 and 15-16 stand rejected as unpatentable over Fahnestock *et al.* in view of Van Eekelen *et al.* As noted above, Van Eekelen *et al.* is not available as a reference. Fahnestock *et al.* alone (or in combination with Van Eekelen *et al.*) does not render obvious the claimed invention. The claimed *Bacilli* are made by the method according to Claim 12 or 13. The *Bacilli* of Claims 15 and 16 are produced according to the method of Claim 12 or 13 (dependent from Claim 12) which specifically recites that the *Bacilli* are obtained by deletion of genes using homologous or illegitimate recombination using the flanking regions of the gene to be deleted. There is no such teaching in Fahnestock *et al.* Accordingly, the Examiner is respectfully requested to withdraw this rejection.

Section 102(b)/103

Claims 12 and 14 stand rejected as anticipated by, or in the alternative as obvious over, Fahnestock *et al*. This rejection is respectfully traversed because Fahnestock *et al*. disclose the inactivation of a protease gene by insertion of a cloned CAT gene; the present invention discloses the inactivation by deletion of an indigenous extracellular protease gene.

Insertion of a CAT gene in an otherwise complete protease gene makes possible the reversion of the inactivation of the protease, whereas in the strains according to the claimed invention, the gene is deleted and reversion is therefore not possible. Further, Claims 12-14 specifically relate to deletion of genes using homologous or illegitimate recombination using the flanking regions of the gene to be deleted, a concept not taught by Fahnestock *et al*. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

No they don't

Priority Claim

Applicants have attached hereto a Supplemental Declaration (unexecuted) claiming priority to U.S. Patent Application 07/162,105 filed February 28, 1988, and to European Application 87200356.1 filed February 27, 1987. An executed Supplemental Declaration and a certified copy of the priority document will be sent under separate cover. Additionally, the claim to U.S. Patent Application 07/162,105 has been added to the information provided under "Cross-Reference to Related Applications" on page 1 of the specification.

Conclusion

In view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the under signed attorney at (415) 494-7622.

Respectfully submitted,

COOLEY GODWARD CASTRO
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TEXT OF PENDING CLAIMS

2. The method according to Claim 21, wherein said *Bacillus strain is an alkalophilic Bacillus* strain.

3. The method according to Claim 22, wherein said DNA sequence encodes a mutant protease.

4. The method according to Claim 23, wherein said *Bacillus* strain is *Bacillus* novo species PB92 or a derivative thereof.

5. The method according to Claim 23, wherein said *Bacillus* strain is an asporogenic alkalophilic *Bacillus* strain.

6. The method according to Claim 23, wherein the gene encoding said native protease has been deleted by homologous or illegitimate recombination.

7. The method according to Claim 23, wherein a plasmid comprises said DNA sequence.

9. The method according to Claim 7, wherein said mutant high alkaline protease is obtained from *Bacillus* novo species PB92.

10. The method according to Claim 23, wherein at least one copy of said DNA sequence is integrated into the genome of said host.

11. The method according to Claim 10, wherein said host further contains at least one copy of a plasmid comprising said DNA sequence.

12. A method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level, said method comprising:

transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5′ and the 3′ flanking regions of a gene coding for a high alkaline protease and wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with an indigenous gene coding for a high alkaline protease whereby transformants are obtained;

growing said transformants under conditions whereby the replication function encoded by said vector is inactivated; and

isolating transformants identified as having a reduced extracellular alkaline protease level.

13. The method according to Claim 12, wherein said alkalophilic *Bacillus* strain is *Bacillus* novo species PB92 or a derivative thereof.

14. An alkalophilic *Bacillus* strain producing a mutant high alkaline protease substantially free of expression product of an indigenous extracellular alkaline protease gene, wherein said strain has been obtained by transforming a mutant alkalophilic *Bacillus* strain having a reduced indigenous extracellular protease level obtained by the method according to Claim 12 or 13 with a plasmid expression vector comprising a mutant high alkaline protease gene.

15. The *Bacillus* strain according to Claim 14, wherein said mutant alkalophilic *Bacillus* strain is a mutant of *Bacillus* novo species PB92 or a derivative thereof.

16. The Bacillus strain according to Claim 15, wherein said indigenous gene has been deleted by homologous or illegitimate recombination.

17. A mutant high alkaline protease produced according to the method of Claim 23 and characterized as (1) substantially free from contamination with a an indigenous extracellular alkaline protease, and (2) differing in at least one amino acid from a wild-type high alkaline protease.

102 reads on any mutated alk. protease (prod. by process)

Cancel Claim 18.

19. A detergent composition comprising as an active ingredient one or more high alkaline protease prepared according to the method of Claim 23.

how mutated?

23. A method for production of a mutated high alkaline protease substantially free of indigenous extracellular protease, said method comprising:

112 1st not all may have this gene to delete.

growing an alkalophilic *Bacillus* strain host having a reduced indigenous extracellular protease level as a result of deletion of the gene for said indigenous extracellular protease

transformed with an expression cassette providing for expression of a mutated high alkaline protease in said host, whereby said mutated high alkaline protease is produced.

24. A method for preparing a detergent composition, which comprises the step of combining a detergent composition with, as an active ingredient, one or more of a high alkaline protease prepared according to the method of Claim 23.

25. A method for processing laundry, which comprises the step of contacting said laundry with a detergent composition comprising as an active ingredient one or more of a high alkaline protease prepared according to the method of Claim 23.

26. A method for production of a mutated high alkaline protease substantially free of indigenous extracellular protease, said method comprising:

growing an asporogenous *Bacillus* strain host having a reduced indigenous extracellular protease level as a result of deletion of the gene for said indigenous extracellular protease transformed with an expression cassette providing for expression of a mutated high alkaline protease in said host, whereby said mutated high alkaline protease is produced.